Canadian Water Quality Guidelines for the Protection of Aquatic Life

CARBAMAZEPINE 2018

Carbamazepine, also known as 5H-Dibenzo[b,f]azepine-5-carboxamide or CBZ, is a tricyclic drug that is commonly prescribed as an antiepileptic. It is also used to treat pain associated with trigeminal neuralgia, and is a psychotropic agent used to treat schizophrenia and bipolar disorder. Combined with other drugs, CBZ may also be effective at treating symptoms of alcohol withdrawal. Most CBZ is used in private settings, with the rest used in hospitals. CBZ is bioactive and of potential concern for aquatic life, especially because some organisms share evolutionarily conserved biochemical pathways with humans.

CBZ enters the aquatic environment mainly through municipal sewage treatment effluent. Other sources include combined sewer overflows during rain events, leakage in sewage systems, introduction into septic systems, application of biosolids onto agricultural land and irrigation with reclaimed water. Disposal in landfills may be a source of concern, but monitoring in Ontario has not found CBZ in landfill leachate to date (Stafford 2008). Some CBZ is manufactured in Canada, but manufacturing releases are probably very low, accounting for about 0.2 to 0.5 percent of product volume (Health Canada 2014).

CBZ is highly resistant to conventional sewage treatment processes but can easily be oxidised by ozone. It has been found in surface water globally, with the highest concentrations often near wastewater treatment effluent outfalls. CBZ is therefore frequently used as a marker of sewage contamination. Surveys in Canada have detected it in influent and effluent, biosolids, surface water, groundwater, treated drinking water, and fish fillets and fish livers, as well as in crops irrigated with reclaimed water. According to Canadian Environmental Protection Act Persistence and Bioaccumulation Regulations (Government of Canada 2000) CBZ is neither bioaccumulative (defined as having a bioaccumulation factor and bioconcentration factor below 5,000 and a log K_{ow} below 5) nor persistent (with a half-life in water of less than 182 days). However, the potential

Table 1. Canadian Water Quality Guideline for the Protection of Aquatic Life for Carbamazepine

	Long-term exposure (µg/L)	Short-term exposure (µg/L)
Freshwater	10*	NRG†
Marine	NRG	NRG

*Type B2 guideline.
†No recommended guideline.

Table 2. Acronyms Used

10-OH-CBZ	10-hydroxy-carbamazepine
CBZ	carbamazepine
CBZ-DiOH	10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine
K_{ow}	octanol/water partition coefficient (a predictor for the distribution of a solute between lipid and aqueous phases)
LOEC	lowest-observed-effect concentration (lowest concentration used in a toxicity test in which toxic effect is significantly different from control concentration)
NOEC	no-observed-effect concentration (highest concentration used in a toxicity test in which toxic effect is not significantly different from control concentration)
EC_{50}	concentration expected to produce a certain effect in 50% of test organisms
LC_{50}	concentration lethal to 50% of test organisms

for continuous presence exists due to CBZ's continual input into surface waters via municipal wastewater effluent.

CBZ is metabolised predominantly in the liver, and at least 30 metabolites have been identified in humans. Five of these have been found in both aqueous and solid phases of wastewater treatment plant processes. In effluent, 10-hydroxy-carbamazepine (10-OH-CBZ) is generally dominant, followed by 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (CBZ-DiOH).

The highest concentration of CBZ found in Canadian surface water is approximately 1 µg/L (Kormos 2007). This concentration (988 ng/L) was detected in a river system in southern Ontario, downstream from 11 sewage treatment plants, and is similar to CBZ concentrations found in European surface water. The highest reported CBZ concentration in Canadian municipal sewage treatment effluent is 2.3 µg/L (Metcalfe *et al.* 2003). The main transformation product (CBZ-DiOH) has also been detected in Canadian surface waters (Otonabee River in Peterborough, Ontario) (Miao and Metcalfe, 2003), often at higher concentrations than CBZ itself (Miao and Metcalfe, 2003; Kase *et al.* 2011). However, this transformation product (CBZ-DiOH) has been identified as biologically inactive (Miao and Metcalfe 2003; Leclercq *et al.* 2009). In contrast, Writer *et al.* (2013) reported that 10-OH-CBZ is the major CBZ metabolite detected in Minnesota surface waters. In any case, CBZ is designed to have pharmacodynamic effects and be bioactive, which is of concern for aquatic receptors (Laville *et al.* 2004; Kim *et al.* 2007), especially when biochemical pathways are evolutionarily conserved among organisms (Gunnarsson *et al.* 2008).

Effects on Freshwater Life

CBZ is somewhat lipophilic, though it expresses both lipophilic and hydrophilic tendencies, and it can cross membranes easily. Once it enters surface water, it may have biological effects, especially if the target receptors in aquatic organisms are similar to those in humans. The sensitivities of invertebrates and fish to short-term CBZ exposure appear to overlap, but invertebrates are the most sensitive. The sensitivities of invertebrates, fish, and aquatic plants and algae to long-term CBZ exposures also overlap, with invertebrates again being the most sensitive (followed by fish, and then aquatic plants and algae). Fish are more able to metabolise CBZ than are invertebrates, which may explain invertebrates' increased sensitivity. Table 3 (next page) summarises the available data for the least and most sensitive fish, invertebrates, and plants and algae for both short-term and long-term exposure.

Table 3. Carbamazepine Toxicity Values for Freshwater Biota,* µg/L

	Short-term				Long-term			
	Most sensitive		Least sensitive		Most sensitive		Least sensitive	
Fish	19,900	*Oncorhynchus mykiss* (rainbow trout) (Li *et al.* 2009) 96-hour LC_{50} (lethality)	86,500	*Danio rerio* (zebrafish) (van den Brandhof and Montforts 2010) 72-hour EC_{50} (growth retardation)	200	*Oncorhynchus mykiss* (Li *et al.* 2009) 42-day NOEC (lower condition factor)	50,000	*Danio rerio* (Ferrari *et al.* 2003, 2004) 10-day LOEC (embryo and larval mortality)
Invertebrates	2,240	*Hydra attenuata* (freshwater polyp) (Quinn *et al.* 2008) 96-hour toxicity threshold (morphological changes)	111,000	*Daphnia magna* (water flea) (Han *et al*.) 48-hour EC_{50} (mobility inhibition)	25/ 100	*Ceriodaphnia dubia* (water flea) (Ferrari *et al.* 2003, 2004) 7-day NOEC/LOEC (reproduction)	2,600	*Chironomus dilutus* (non-biting midge) (Dussault *et al*. 2008) 10-day EC_{10} (growth)
					10/ 100	*Daphnia pulex* (water flea) (Lürling *et al.* 2006) 14-day NOEC/LOEC (nominal survival)		
Plants and algae	110,929	*Chlorella vulgaris* (green algae) (Jos *et al.* 2003) 24-hour EC_{50} (growth inhibition)	n/a	none available	500	*Chlorella pyrenoidosa* (green algae) (Zhang *et al.* 2012) 96-hour NOEC (growth inhibition)	74,000	*Desmodesmus subspicatus* (green algae) (Cleuvers 2003) 72-hour EC_{50} (growth inhibition)

* CCME found only one study (Richards and Cole 2006) that investigated the toxicity of CBZ to an amphibian, and this study did not produce any signs of malformation or mortality at the highest test concentrations (100,000 µg/L).

The highest CBZ concentration found in Canadian surface water (approximately 1 µg/L) and the toxicity data presented above together indicate that acute toxicity to aquatic life is unlikely. Due to the continuous presence of CBZ in the aquatic environment, combined with the fact that the chronic LOECs (*Ceriodaphnia dubia* [Ferrari *et al*. 2003, 2004] and *Daphnia pulex* [Lürling *et al.* 2006]) are within an order of magnitude of the highest detected surface water concentrations, chronic toxicity is more likely than acute toxicity. Potential also exists for unknown mixture effects involving CBZ or its transformation products and other chemicals. Of additional concern is co-exposure to other pharmaceuticals with similar modes of action (such as sedation or anxiety reduction), which may not alone be toxic.

Uncertainty still surrounds the toxicity of CBZ transformation products. The Canadian Council of Ministers of the Environment (CCME) found one study (Bernhard 2010; SMDS 2011) that investigated the toxicity of the transformation product CBZ-DiOH to two *Daphnia* species. This study found a 28-day LOEC value of ≤0.5 µg/L for increased number of offspring (*D. magna* only) and size changes in offspring (a decrease for *D. magna* and an increase for *D. cucullata*).

Information is scarce concerning the influence of factors such as pH and temperature on the toxicity of CBZ. One study (Meredith-Williams 2012) noted that many pharmaceuticals are ionisable. Studies on fish, daphnids and plants with a variety of drugs have shown that pH changes in the environment easily affect uptake and resulting toxicity of ionisable pharmaceuticals (Zhang *et al.* 2008; Moermond 2014). However, CBZ is not expected to ionise at environmentally relevant pH ranges.

Water Quality Guideline Derivation

CCME could not derive a short-term freshwater benchmark concentration from available primary data (CCME 2007). There is a paucity of acceptable studies for invertebrates, even for deriving a Type B2 benchmark concentration. The minimum dataset requirement of endpoints for two aquatic or semi-aquatic species (which must come from primary studies) was not fulfilled. A similar lack of acceptable studies holds true for fish. The minimum dataset requirement of endpoints for two species of fish (at least one salmonid and one non-salmonid, both of which must come from primary studies) was also not fulfilled. Furthermore, most published short-term toxicity studies with CBZ used solvent to keep CBZ dissolved in solution, resulting in effect concentrations that were above the compound's natural solubility in water. Based on this, CCME concludes that CBZ is unlikely to have acute toxic effects on aquatic organisms. The highest concentration of CBZ found in Canadian surface waters (1 ug/L) is about four orders of magnitude below any concentration causing acute toxicity. Therefore, short-term effects are highly unlikely to occur in Canada.

CCME has developed a long-term freshwater Canadian water quality guideline for CBZ. Most of the available long-term toxicity studies measured different endpoints (e.g., molecular markers and results from in vitro assays) than are normally used in developing Canadian water quality guidelines (e.g., survival, growth, reproduction). Based on available data, CCME developed a long-term freshwater Type B2 (deterministic) guideline using the critical toxicity value plus assessment factor approach. To derive a Type B2 long-term exposure guideline, CCME uses the lowest acceptable endpoint—that is, the most sensitive preferred LOEC endpoint—found in any long-term exposure study. A study classified as secondary is acceptable. The endpoint concentration from this critical study is then divided by a safety factor of 10 to derive a long-term exposure guideline value. The critical study, on *C. dubia*, reported a seven-day NOEC and LOEC of 25 µg/L and 100 µg/L, respectively, for significant decreases in reproduction compared to controls. Per protocol, Type B2 long-term guidelines are derived using LOEC data. Applying a

safety factor of 10 to the LOEC of 100 µg/L results in a Canadian Water Quality Guideline of 10 µg/L.

Traditionally, additional data from standard toxicity tests (which assess survival, growth and reproduction) would be required to fill in data gaps in order to upgrade the Type B2 guideline to a Type B1 (deterministic) or Type A (probabilistic) value. In the case of low-level exposures to pharmaceuticals such as CBZ, standard chronic toxicity tests may not utilize endpoints adequate for assessing specific effects. Pharmaceuticals can elicit low-dose effects due to being designed for biological activity. Perhaps more sensitive and specific endpoints would be more useful (e.g., evidence is growing that environmentally relevant concentrations of pharmaceuticals can elicit changes in behaviour [CCME 1999]).

Following the CCME protocol, CCME considered only studies that provide data for standard endpoints such as survival, growth and reproduction. Some researchers (Triebskorn *et al.* 2007) argue that standard toxicity tests do not measure endpoints sensitive enough to detect impacts of pharmaceuticals, as such tests do not account for specific modes of pharmaceutical action. Many toxicity studies for CBZ reported non-standard endpoints such as altered enzyme activity. Some also conducted histological and cytological investigation of the liver, kidney and gills, or conducted transcriptomics. These studies are detailed in the scientific criteria document (CCME 2018), with some effect concentrations below the long-term exposure Canadian Water Quality guideline value of 10 µg/L.

Data were insufficient to derive short- or long-term guidelines for the protection of marine life.

Implementation and Other Considerations

This guideline is designed to provide protection for long-term exposure periods. It is a conservative value below which CCME expects all forms of aquatic life, during all life stages and in all Canadian aquatic systems, to be protected. This guideline has not been corrected for any toxicity-modifying factors. Exceeding the guideline does not necessarily suggest that toxic effects will occur, but rather indicates the need to determine whether or not there is a potential for adverse environmental effects.

The guideline should be used as a screening and management tool to ensure that CBZ does not lead to the degradation of the aquatic environment.

The guideline does not consider concentrations of transformation products. Transformation products may contribute to toxicity, or they may be converted back into the parent compound in the environment (Bahlmann *et al.* 2014). The science related to toxicity and fate of transformation products is not yet developed and therefore cannot be included in derivation of the guideline.

If the toxicity of the transformation products is found to be similar to or greater than that of CBZ, these compounds may be of similar or even greater concern than the parent product. There is currently active discussion in the literature concerning whether parent compounds and transformation products are equally important for consideration in risk assessments, because these mixtures may show additive or synergistic toxicity. Until more is known in this regard, CCME will use the same approach as is used for pesticides, where only the parent compound is considered.

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